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The Effect of Green Tea Leaf Extract on Reproductive Function of Male Wistar Rats

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ABSTRACT

This study assessed the effects green leaf tea extract on male reproductive function of male albino wistar rats. Twenty rats weighing between 150- 250g, grouped into 4 of 5 rats each, were used for the research that lasted for four weeks. Group I, the control group, received normal rat chow and water ad libitum. The three test groups II-IV, received 2.5gm/100ml 0f water, 5gm/100ml of water, 7.5gm/100ml of water of green leaf tea extract given via oral administration with normal rat food and water for four weeks. Blood was collected via ocular puncture and serum was assayed for testosterone levels. Semen was also analyzed for sperm motility and sperm count. The testicular weight was recorded and net body weight gain was also recorded. The study showed significant decrease in net body weight gain and testicular weight. It has been shown that the reduction of body weight after application of green tea extract may be due to inhibition of catechol-O-methyl transferase transferase (COMT) enzyme by epigallocatechin gallate (EGCG) of the green tea. The study also showed significant decrease in sperm motility and sperm count. It also showed a significant decrease in testosterone levels. In conclusion, green leaf tea extract had a negative effect on male reproductive function and its use as a daily tea should be curtailed.

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1. BACKGROUND OF STUDY

Male infertility refers to the inability of a man to impregnate a woman after 12 months of regular and unprotected sexual intercourse (Emokpae, 1999).Since the 1990s, there have been reports about decreasing human sperm counts and increasing abnormalities in human testes which have focused research on the possible impact of environmental factors such as life style, pollution, occupational exposure to trace elements and pesticides.

The World Health Organization in 1991 estimated that, 8–12% of couples worldwide experienced some forms of infertility during their reproductive lives, thus affecting 50–80 million couples with 20–35 million in Africa (W.H.O infertility, 1991)

3–4 million Nigerian couples are affected (Thomas, *et al.,* 1995; Sule, *et al*., 2008). Even though infertility is not lethal, it has been described as a radical life changing problem that carries with it significant psychological trauma. Male factor infertility is responsible for about 40–50% of all infertility cases.

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea.

Green is nature's treasure to mankind, next to water as the most consumed beverage in the world (Gomikawa *et al*., 2008).It is obtained from the tea plant Camellia sinensis which belongs to the family Theacaeae and is cultivated in at least 30 countries around the world, commonly consumed in Japan, China, India and other Asian countries, some parts of North Africa, United State and Europe (Namita *et al*., 2012).

1.1. SIGNIFICANCE OF STUDY

It has been demonstrated earlier on that green tea leaf extract has significant role in decrease in testosterone level as well as changes in morphological character of testis (Smith & Dou, 2001).

1.2. AIM OF STUDY

This study was carried out to investigate the effect of green tea on male reproduction function of male albino wistar rat.

1.3. OBJECTIVES

- The specific objectives are:

To determine the effect green tea on gained body weight, testicular weight, sperm motility, sperm count and testosterone levels.

2. LITERATURE REVIEW

Green tea is the nature's treasure to mankind, next to water as the most consumed beverage in the world (Gomikawa *et al.,* 2008). It is obtained from the tea plant Camellia sinensis which belongs to the family Theaceae and is cultivated in at

least 30 countries around the world, commonly consumed in Japan, China, India and other Asian countries, some parts of North Africa, the United States, and Europe (Maruyama et al., 2009; Chacko *et al*., 2010; Namita *et al.,* 2012). Green tea is produced by inactivating the heat-labile enzyme polyphenol oxidase in the fresh leaves by either applying heat or steam, which prevents the enzymatic oxidation of catechins, the most abundant flavonoid compounds present in green tea extracts (Velayutham *et al*., 2008). The chemical composition of green tea varies with climate, season, horticultural practices, and the position of the leaf on the harvested shoot (Pastore, 2005). The active constituents in green tea are powerful antioxidants called polyphenols. Tea is reported to contain nearly 4000 bioactive compounds of which one third is contributed by polyphenols (Tariq *et al.,* 2010). Among the polyphenols in tea, is a family of compounds called the flavonoids? Flavanoids (and their fraction, catechins) are the basic phenolic compounds in green tea responsible for antioxidant activities such as neutralization of free radicals that are formed in the process of metabolism (Horzic *et al*., 2009). These flavanoids contain a substance called catechins. Major catechins present in green tea are epicatechin (EC), epigallocatechingallate (EGCG), epigallocatechins (EGC) and epicatechingallate (ECG). The history of the medical effects of green tea starts on the early eighth century with the Buddhist monks who recognized green tea for its medicinal powers; therefore nowadays there is also an increasing interest in the beneficial effects of green tea on disease prevention (Neves *et al.,* 2010). Its active components are reported to have several biological properties, including cancer chemoprevention, inhibition of tumor cell growth, antiviral and anti-inflammatory activities (Yang *et al.,* 2000), antioxidant activity (Morel *et al*., 1993; Guo *et al*., 1996), antimutagenic and anticlastogenic effects (Gupta et al., 2002) and inhibitory effects on several enzymes, such as aromatase (Satoh et al., 2002), angiotensin converting enzyme (Actis-Goretta *et al.,* 2006) and thyroid peroxidase (Divi and Doerge, 1996). Also diminish 002 E3 J. Med. Res. the risk of different illnesses, including diabetes, cancer and coronary heart disease (Dufresne and Farnworth, 2001; Chaiyasut *et al.,* 2011)

Several plants are reported to enhance reproductive ability and some are known to hamper such functions. Ethanolic extract of Semecarpusanacardium fruit (Sharma *et al*., 2003) causes spermatogenic arrest in male albino rats. Oral administration of plumieride (Gupta *et al.,* 2004) causes spermatogenic arrest without any noticeable side effects. Hydro-methanolic extract of leaf of Aeglemermelos (Das *et al*., 2009) has antigonadal effect in male rats. Tulsi (Oscimum sanctum) (Kashinathan *et al.,* 2004) and (Kashinathan *et al.,* 1996) are antifertility agents while after ginger (Zingiberofficinale) (Khaki *et al.,* 2009) administration causes accumulation of sperms in the lumen of seminiferous tubule. It has been demonstrated that methanolic pod extract of Albizzialebbeck (L) Benth (Gupta *et al.,* 2004) has anti spermatogenic activity. Green tea components theanine and catechin have reproductive effects (Yokogoshi et al., 2004). It has been demonstrated earlier that Aliumsativum (Chakraborty *et al.,* 2003) bulb extract has its spermicidal activities. Sarcostemaacidum (Venma *et al*., 2002) stem extract exhibit spermatogenic arrest in male rats without any side effects. It has been reported that there was a reduction in plasma testosterone level by epigallocatechingallate present in green tea (Kao *et al.,* 2000). It has been demonstrated earlier on that green tea leaf extract has significant role in decrease in testosterone level as well as changes in morphological character of testis (Chandra *et al*., 2011). Green tea catechin has been shown to inhibit tumor cell proliferation and promote the destruction of leukemia cell (Smith *et al*., 2001) and breast cancer cells (Vergote *et al*., 2002). Green tea was also shown to decrease the risk of developing ovarian cancer (Zhang *et al*., 2002). It has been suggested that excessive intake of tea should have been avoided by those people who are prone to anaemia (Samman *et al*., 2001). The present study was undertaken to evaluate the morphological and functional changes in testis as well as hormonal level by administration of green tea leaf extract.

A new study has found that an extract from green tea affects sperm quality. The research, published last month in the journal Molecular Nutrition and Food Research, found that low doses of a chemical compound (epigallocatechingallate or EGCG) which is present in green tea can improve sperm quality.

Sub-fertility among men is common, and numbers of men affected are increasing. Recent data suggests that 1 in 5 men between the ages of 18-25 now have fertility problems linked to semen quality (2). In around 50% of cases, the cause of male subfertility is unknown, and in such cases nutritional and lifestyle measures are often recommended as a means of boosting sperm quality.

In this recent study, researchers exposed human sperm samples to a range of concentrations of EGCG, a chemical compound present in green tea. Results showed that, at low concentrations, EGCG was associated with increased sperm motility, viability, and phosphorylation of proteins controlling cell survival.

Previous studies support the value of antioxidants in boosting male fertility (Chakraborty *et al.,* 2003). The high antioxidant value of green tea is well known, and this characteristic may therefore play a role in its fertility-boosting potential. Sperm damage is thought to occur when highly reactive particles called free radicals circulate in the body, causing damage to sperm cells. This damage may reduce fertility by lowering sperm counts or reducing the sperm's ability to fertilize an egg. For this reason, antioxidants, which fight those free radicals, are thought to be helpful.

Further controlled trials are certainly needed to provide solid guidelines on the benefits of nutrients in treating male fertility. My feeling is that further research will serve to confirm the crucial role for diet and lifestyle in this area. The European Science Foundation recently reported new figures showing a rapid increase in male reproductive disorders. This indicates that these fertility issues are caused by environmental factors or changes in our lifestyle rather than genetic factors, meaning that they may be entirely preventable with the natural approach focusing on nutrition and lifestyle (De-Amicis *et al.,* 2012; Showell *et al* 2011).

2.1. Green tea

Tea is one of the most popular beverages consumed worldwide. Tea, from the plant *Camellia sinensis*, is consumed in different parts of the world as green, black, or Oolong tea. Among all of these, however, the most significant effects on human health have been observed with the consumption of green tea (Chakraborty *et al.,* 2003). The first green tea was exported from India to Japan during the

17th century. It is estimated that about 2.5 million tons of tea leaves are produced each year throughout the world, with 20% produced as green tea, which is mainly consumed in Asia, some parts of North Africa, the United States, and Europe. The association between tea consumption, especially green tea, and human health has long been appreciated (Chakraborty *et al.,* 2003). Green tea and black tea are processed differently during manufacturing. To produce green tea, freshly harvested leaves are immediately steamed to prevent fermentation, yielding a dry, stable product. This steaming process destroys the enzymes responsible for breaking down the color pigments in the leaves and allows the tea to maintain its green color during the subsequent rolling and drying processes. These processes preserve natural polyphenols with respect to the health-promoting properties. As green tea is fermented to Oolong and then to black tea, polyphenol compounds (catechins) in green tea are dimerized to form a variety of theaflavins, such that these teas may have different biological activities.

2.2. Green tea composition

The chemical composition of green tea is complex: proteins (15-20% dry weight), whose enzymes constitute an important fraction; amino acids (1-4% dry weight) such as theanine or 5-*N*-ethylglutamine, glutamic acid, tryptophan, glycine, serine, aspartic acid, tyrosine, valine, leucine, threonine, arginine, and lysine; carbohydrates (5-7% dry weight) such as cellulose, pectins, glucose, fructose, and sucrose; minerals and trace elements (5% dry weight) such as calcium, magnesium, chromium, manganese, iron, copper, zinc, molybdenum, selenium, sodium, phosphorus, cobalt, strontium, nickel, potassium, fluorine, and aluminum; and trace amounts of lipids (linoleic and α-linolenic acids), sterols (stigmasterol), vitamins (B, C, E), xanthic bases (caffeine, theophylline), pigments (chlorophyll, carotenoids), and volatile compounds (aldehydes, alcohols, esters, lactones, hydrocarbons). Due to the great importance of the mineral presence in tea, many studies have determined their levels in tea leaves and their infusions(Table1). Fresh leaves contain, on average, 3-4% of alkaloids known as methylxanthines, such as caffeine, theobromine, and theophylline (Showell *et al.,* 2011). In addition, there are phenolic acids such as gallic acids and characteristic amino acid such as theanine present (Showell *et al.,* 2011)

Table 1 Composition (%) of green tea, black tea, and black tea infusion

Compound	Green Tea*	Black tea*	Infusion*
Protein	15	15	trace
Amino acids	4	4	3.5
Fiber	26	26	0
Others carbohydrates	7	7	4
Lipids	7	7	trace
Pigments	2	2	trace
Minerals	5	5	4.5
Phenolic compounds‡	30	5	4.5
Oxidized phenolic compounds§	0	25	4.5

Green tea contains polyphenols, which include flavanols, flavandiols, flavonoids, and phenolic acids; these compounds may account for up to 30% of the dry weight. Most of the green tea polyphenols (GTPs) are flavonols, commonly known as catechins. Products derived from green tea are mainly extracts of green tea in liquid or powder form that vary in the proportion of polyphenols (45-90%) and caffeine content (0.4-10%). The major flavonoids of green tea are various catechins, which are found in greater amounts in green tea than in black or Oolong tea (Gomikawa, *et al*., 2008) There are four kinds of catechins mainly find in green tea: epicatechin, epigallocatechin, epicatechin-3-gallate, and EGCG (Chacko, *et al*., 2014). The preparation methods influence the catechins both quantitatively and qualitatively; the amount of catechins also varies in the original tea leaves due to differences in variety, origin, and growing conditions (Maruyama,*et al*.,2009). The preparation of fresh green tea cannot totally extract catechins from the leaves; therefore, the concentration found differs from the absolute values determined through the complete extraction of leaves (Namita, *et al.,* 2005). Moreover, catechins are relatively unstable and could be quantitatively and qualitatively modified during the time frame of an experiment Pastore (2005). Thus, comparison of ingested doses in animal studies is not possible because the catechin quantification before administration is often not known.

2.3. Health benefits of green tea in humans and animals

Studies using animal models show that green tea catechins provide some protection against degenerative diseases (Yang CS,et al;2000). Some studies indicated that green tea has an antiproliferative activity on hepatoma cells and a hypolipidemic activity in hepatoma-treated rats, as well as the prevention of hepatoxicity (Yang *et al.,* 2000) and as a preventive agent against mammary cancer post-initiation (Yang, *et al*., 2000) Green tea catechins could also act as antitumorigenic agents (Morel *et al.,* 1993) and as immune modulators in immunodysfunction caused by transplanted tumors or by carcinogen treatment (Yang *et al*., 2000). Moreover, green tea, its extract, and its isolated constituents were also found to be effective in preventing oxidative stress and neurological problems (Guo *et al*., 1996)

Green tea consumption has also been linked to the prevention of many types of cancer, including lung, colon, esophagus, mouth, stomach, small intestine, kidney, pancreas, and mammary glands (Guo, 1996) .Several epidemiological studies and clinical trials showed that green tea (and black and Oolong teas to a lesser extent) may reduce the risk of many chronic diseases (Gupta, *et al.,* 2002). This beneficial effect has been attributed to the presence of high amounts of polyphenols, which are potent antioxidants. In particular, green tea may lower blood pressure and thus reduce the risk of stroke and coronary heart disease. Some animal's studies suggested that green tea might protect against the development of coronary heart disease by reducing blood glucose levels and body weight (Satoh, *et al.,* 2002). However, all these data are based on middle-aged animals' populations, not the elderly populations, which nutritional status tends to be more adversely influenced by age-related biological and socioeconomic factors (Actis-Goretta *et al* 2006)

Tea components possess antioxidant, antimutagenic, and anticarcinogenic effects and could protect humans against the risk of cancer by environmental agents (Divi, *et al*., 2006) Sano *et al* , reported the inhibitory effects of green tea leaves against tert-butyl hydroperoxide-induced lipid peroxidation, and a similar antioxidant effect on the kidney was observed after oral administration of the major tea polyphenol EGCG. The antioxidative potency of crude catechin powder and

individual catechins was tested in experiments using the active oxygen method. Crude catechins reduced the formation of peroxides far more effectively than dl-α-tocopherol (Chaiyasut, *et al*., 2011) Shim *et al*. Studied the chemopreventive effect of green tea among cigarette smokers and found that it can block the cigarette-induced increase in sister chromatid exchange frequency.

The effectiveness of green tea in treating any type of diarrhea and typhoid has been known in Asia since ancient times (Morel, *et al*., 1993) Green tea catechins have an inhibitory effect on *Helicobacter pylori* infection (Namita *et al*., 2012) Effects of green tea against the influenza virus, especially in its earliest stage, as well as against the *Herpes simplex* virus have also been demonstrated (Neves *et al.,* 2010) Furthermore, Weber *et al*. observed that adenovirus infection is inhibited *in vitro* by green tea catechins.

In humans, Hirasawa and Takada studied the antifungal activity of green tea catechins against *Candida albicans* and the convenience of a combined treatment with catechins and lower doses of antimycotics, which may help to avoid the side effects of antimycotics. Green tea consumption has also been associated with increased bone mineral density, and it has been identified as an independent factor protecting against the risk of hip fractures; this effect was considered independent of smoking status, hormone replacement therapy, coffee drinking, and the addition of milk to tea Pastore, (2005). Park *et al*. Observed the positive effects of green tea extracts and GTPs on the proliferation and activity of bone cells. The proliferation of hepatic stellate cells is closely related to the progression of liver fibrosis in chronic liver diseases, and EGCG has a potential inhibitory effect on the proliferation of these cells Paul (2008). Green tea strengthens the immune system action because it protects it against oxidants and radicals. Recent studies suggested that GTPs might protect against Parkinson's and Alzheimer's diseases and other neurodegenerative diseases (Rezk*, et al*., 2014). Studies have demonstrated GTP neuroprotectant activity in cell cultures and animal models, such as the prevention of neurotoxin-induced cell injury (Rezk, *et al*., 2014). Green tea is considered to be useful for insect stings due mainly to its anti-inflammatory effects and its capacity to stop bleeding (Roshdy, *et al*., 2013) some studies have suggested an inverse association between green tea consumption and the risk of kidney stone formation. In an experimental cataractogenesis system, green tea acted by preserving the antioxidant defense system of the lens (Roshdy *et al*., 2013). Skrzydlewska *et al*. indicated a beneficial effect of green tea in alcohol intoxication. In addition to all of these reported properties, which have helped the recognition of green tea as functional food by some authors (Sueoka *et al*., 2010) green tea is also currently used in the preparation of a variety of foods, pharmaceutical preparations, dentifrices, and cosmetics (Satoh, *et al*., 2002)

Tea has been shown anticarcinogenic effects against breast cancer in experimental studies (Satoh K,et al ;2002). However, epidemiologic evidence that tea protects against breast cancer has been inconsistent (Satoh, *et al*., 2002)

A case-control study was conducted in southeastern China between 2004 and 2005 (Sha'bani *et al.,* 2015). The incidence cases were 1009 female patients aged 20-87 years with histologically confirmed breast cancer, and the 1009 age-matched controls were healthy women randomly recruited from breast disease clinics. Information on duration, frequency, quantity, preparation, and type of tea consumption as well as diet and lifestyle were collected by face-to-face interviews using a validated and reliable questionnaire. In comparison with non-tea drinkers, green tea drinkers tended to reside in urban settings, to have more education, and to consume more coffee, alcohol, soy, vegetables, and fruits. After adjusting established and potential confounding factors, green tea consumption was associated with a reduced risk of breast cancer. Similar dose-response relationships were observed for duration of drinking green tea, number of cups consumed, and new batches prepared per day.

Hsu *et al*. demonstrated the effects of supplementation with decaffeinated green tea extract (catechins) on hemodialysis-induced reactive oxygen species, atherosclerotic disease risk factors, and proinflammatory cytokines. The pharmacokinetics of one oral dose of catechins was compared between healthy subjects and hemodialysis patients. The authors compared the antioxidant effects of three different doses (0, 455, and 910 mg) of oral catechins with that of oral vitamin C (500 mg) during a hemodialysis session. In patients, catechin supplementation reduced hemodialysis-enhanced plasma hypochlorous acid activity more effectively than did placebo or vitamin C. Between the treatments with 455 and 910 mg catechins, no significant difference was found in the reduction of plasma hypochlorous acid activity. Catechins also significantly reduced proinflammatory cytokine expression enhanced by hemodialysis.

2.3.1. OVERVIEW OF MALE REPRODUCTIVE SYSTEM & FUNCTION

In simple terms, reproduction is the process by which organisms create descendants. This miracle is a characteristic that all living things have in common and sets them apart from nonliving things. But even though the reproductive system is essential to keeping a species alive, it is not essential to keeping an individual alive (Valerie, *et al.*, 2017).

In human reproduction, two kinds of sex cells or gametes are involved. Sperm, the male gamete, and a secondary oocyte (along with first polar body and corona radiata), the female gamete must meet in the female reproductive system to create a new individual. For reproduction to occur, both the female and male reproductive systems are essential. It is a common misnomer to refer to a woman's gametic cell as an egg or ovum, but this is impossible. A secondary oocyte must be fertilized by the male gamete before it becomes an "ovum" or "egg".

While both the female and male reproductive systems are involved with producing, nourishing and transporting either the oocyte or sperm, they are different in shape and structure. The male has reproductive organs, or genitals, that are both inside and outside the pelvis, while the female has reproductive organs entirely within the pelvis.

The male reproductive system consists of the testes and a series of ducts and glands. Sperm are produced in the testes and are transported through the reproductive ducts. These ducts include the epididymis, vas deferens, ejaculatory duct and urethra. The reproductive glands produce secretions that become part of semen, the fluid that is ejaculated from the urethra. These glands include the seminal vesicles, prostate gland, and bulbourethral glands (Valerie, *et al.,* 2017).

Testes: The testes (singular, testis) are located in the scrotum (a sac of skin between the upper thighs). In the male fetus, the testes develop near the kidneys, then descend into the scrotum just before birth. Each testis is about 1 1/2 inches long by 1 inch wide. Testosterone is produced in the testes which stimulates the production of sperm as well as give secondary sex characteristics beginning at puberty.

Scrotum: The two testicles are each held in a fleshy sac called the scrotum. The major function of the scrotal sac is to keep the testes cooler than thirty-seven degrees Celsius (ninety-eight point six degrees Fahrenheit). The external appearance of the scrotum varies at different times in the same individual depending upon temperature and the subsequent contraction or relaxation of two muscles. These two muscles contract involuntarily when it is cold to move the testes closer to the heat of the body in the pelvic region. This causes the scrotum to appear tightly wrinkled. On the contrary, they relax in warm temperatures causing the testes to lower and the scrotum to become flaccid. The temperature of the testes is maintained at about thirty-five degrees Celsius (ninety-five degrees Fahrenheit), which is below normal body temperature. Temperature has to be lower than normal in order for *spermatogenesis* (sperm production) to take place. The two muscles that regulate the temperature of the testes are the dartos and cremaster muscles:

- **Dartos Muscle**

The dartos muscle is a layer of smooth muscle fibers in the subcutaneous tissue of the scrotum (surrounding the scrotum). This muscle is responsible for wrinkling up the scrotum, in conditions of cold weather, in order to maintain the correct temperature for spermatogenesis.

- **Cremaster Muscle**

The cremaster muscle is a thin strand of skeletal muscle associated with the testes and spermatic cord. This muscle is a continuation of the internal oblique muscle of the abdominal wall, from which it is derived.

Seminiferous Tubules: Each testis contains over 100 yards of tightly packed seminiferous tubules. Around 90% of the weight of each testes consists of seminiferous tubules. The seminiferous tubules are the functional units of the testis, where spermatogenesis takes place. Once the sperm are produced, they moved from the seminiferous tubules into the rete testis for further maturation.

Interstitial Cells (Cells of Leydig): In between the seminiferous tubules within the testes, are instititial cells, or, *Cells of Leydig*. They are responsible for secreting the male sex hormones (i.e., testosterone).

Sertoli Cells: A Sertoli cell (a kind of sustentacular cell) is a 'nurse' cell of the testes which is part of a seminiferous tubule. It is activated by follicle-stimulating hormone, and has FSH-receptor on its membranes. Its main function is to nurture the developing sperm cells through the stages of spermatogenesis. Because of this, it has also been called the "mother cell." It provides both secretory and structural support. Other functions During the Maturation phase of spermiogenesis, the Sertoli cells consume the unneeded portions of the spermatazoa.

Efferent ductules: The sperm are transported out of the testis and into the epididymis through a series of efferent ductules.

Blood Supply: The testes receive blood through the testicular arteries (gonadal artery). Venous blood is drained by the testicular veins. The right testicular vein drains directly into the inferior vena cava. The left testicular vein drains into the left renal vein.

Epididymis: The seminiferous tubules join together to become the epididymis. The epididymis is a tube that is about 2 inches that is coiled on the posterior surface of each testis. Within the epididymis the sperm complete their maturation and their flagella become functional. This is also a site to store sperm, nourishing them until the next ejaculation. Smooth muscle in the wall of the epididymis propels the sperm into the ductus deferens. Vasa efferentia from the rete testis open into the epididymis which is a highly coiled tubule. The epididymis has three parts-

1. Head or caput epididymis- it is the proximal part of the epididymis. It carries the sperms from the testis.
2. Body or corpus epididymis- it the highly convoluted middle part of the epididymis
3. Tail or cauda epididymis- it is the last part that takes part in carrying the sperms to the vas deferens. The cauda epididymis continues to form less convoluted vas deferens (Valerie, *et al.,* 2017).

Ductus Deferens: The ductus (vas) deferens, also called sperm duct, or, spermatic deferens, extends from the epididymis in the scrotum on its own side into the abdominal cavity through the inguinal canal. The inguinal canal is an opening in the abdominal wall for the spermatic cord (a connective tissue sheath that contains the ductus deferens, testicular blood vessels, and nerves. The smooth muscle layer of the ductus deferens contracts in waves of peristalsis during ejaculation.

Seminal Vesicles: The pair of seminal vesicles are posterior to the urinary bladder. They secrete fructose to provide an energy source for sperm and alkalinity to enhance sperm mobility. The duct of each seminal vesicle joins the ductus deferens on that side to form the ejaculatory duct.

Ejaculatory Ducts: There are two ejaculatory ducts. Each receives sperm from the ductus deferens and the secretions of the seminal vesicle on its own side. Both ejaculatory ducts empty into the single urethra.

Prostate Gland: The prostate gland is a muscular gland that surrounds the first inch of the urethra as it emerges from the bladder. The smooth muscle of the prostate gland contracts during ejaculation to contribute to the expulsion of semen from the urethra.

Bulbourethral Glands: The bulbourethral glands also called Cowper's glands are located below the prostate gland and empty into the urethra. The alkalinity of seminal fluid helps neutralize the acidic vaginal pH and permits sperm mobility in what might otherwise be an unfavorable environment.

Penis: The penis is an external genital organ. The distal end of the penis is called the glans penis and is covered with a fold of skin called the prepuce or foreskin. Within the penis are masses of erectile tissue. Each consists of a framework of smooth muscle and connective tissue that contains blood sinuses, which are large, irregular vascular channels.

Urethra: The urethra, which is the last part of the urinary tract, traverses the corpus spongiosum and its opening, known as the meatus, lies on the tip of the glans penis. It is both a passage for urine and for the ejaculation of semen.

2.3.2. COMPOSITION OF HUMAN SEMEN

The components of semen come from two sources: sperm, and "seminal plasma". Seminal plasma, in turn, is produced by contributions from the seminal vesicle, prostate, and bulbourethral glands. Seminal plasma of humans contains a complex range of organic and inorganic constituents. The seminal plasma provides a nutritive and protective medium for the spermatozoa during their journey through the female reproductive tract. The normal environment of the vagina is a hostile one for sperm cells, as it is very acidic (from the native microflora producing lactic acid), viscous, and patrolled by immune cells. The components in the seminal plasma attempt to compensate for this hostile environment. Basic amines such as putrescine, spermine, spermidine and cadaverine are responsible for the smell and flavor of semen. These alkaline bases counteract the acidic environment of the vaginal canal, and protect DNA inside the sperm from acidic denaturation (Valerie, *et al.,* 2017).

GLAND	APPROXIMATE %	DESCRIPTION
Testes	2-5%	Approximately 200- to 500-million spermatozoa (also called sperm or spermatozoans), produced in the testes, are released per ejaculation
seminal vesicle	65-75%	amino acids, citrate, enzymes, flavins, fructose (the main energy source of sperm cells, which rely entirely on sugars from the seminal plasma for energy), phosphorylcholine, prostaglandins (involved in suppressing an immune response by the female against the foreign semen), proteins, vitamin C
Prostate	25-30%	acid phosphatase, citric acid, fibrinolysin, prostate specific antigen, proteolytic enzymes, zinc (serves to help to stabilize the DNA-containing chromatin in the sperm cells. A zinc deficiency may result in lowered fertility because of increased sperm fragility. Zinc deficiency can also adversely affect spermatogenesis.)
bulbourethral glands	< 1%	galactose, mucus (serve to increase the mobility of sperm cells in the vagina and cervix by creating a less viscous channel for the sperm cells to swim through, and preventing their diffusion out of the semen. Contributes to the cohesive jelly-like texture of semen.), pre-ejaculate, sialic acid

A 1992 World Health Organization report described normal human semen as having a volume of 2 ml or greater, pH of 7.2 to 8.0, sperm concentration of 20x106 spermatozoa/ml or more, sperm count of 40x106 spermatozoa per ejaculate or more and motility of 50% or more with forward progression (categories a and b) of 25% or more with rapid progression (category a) within 60 minutes of ejaculation (WebMD, 2017)

2.3.4. HORMONE REGULATION

Hormones which control reproduction in males are:

Gonadotropin-Releasing Hormone (GnRH):

- The hypothalamus secretes this hormone into the pituitary gland in the brain.
- There are two gonadotropic hormones, FSH and LH.
- Luteinizing Hormone (LH):
- The pituitary gland secretes this hormone after receiving a GnRH signal from the hypothalamus.
- LH stimulates Leydig cells, in the testes, telling them to produce testosterone.
- Follicle-Stimulating Hormone (FSH):
- The pituitary gland also secretes this hormone.
- Testosterone helps FSH run through the bloodstream to make Sertoli cells, located in the seminiferous tubules of the testes, to make immature sperm to mature sperm.

Testosterone:

- Also known as "the male hormone" and "androgen".

Testosterone is vital for the production of sperm

Erection: The erection of the penis is its enlarged and firm state. It depends on a complex interaction of psychological, neural, vascular and endocrine factors. The term is also applied to the process that leads to this state.A penile erection occurs when two tubular structures that run the length of the penis, the corpora cavernosa, become engorged with venous blood. This is a result of parasympathetic nerve induced vasodilation (WebMD, 2017). This may result from any of various physiological stimuli. The corpus spongiosum is a single tubular structure located just below the corpora cavernosa, which contains the urethra, through which urine and semen pass during urination and ejaculation, respectively. This may also become slightly engorged with blood, but less so than the corpora cavernosa. Penile erection usually results from sexual stimulation and/or arousal, but can also occur by such causes as a full urinary bladder or spontaneously during the course of a day or at night, often during erotic or wet dreams. An erection results in swelling and enlargement of the penis. Erection enables sexual intercourse and other sexual activities (sexual functions), though it is not essential for all sexual activities.

Transverse section of the penis.

Ejaculation: Emission is the term used when sperm moves into the urethra. Ejaculation is the term used when sperm is forced out of the urethra and the penis. These are both stimulated by sympathetic nerves.

Sperm Production: A spermatozoon or spermatozoan (pl. spermatozoa), from the ancient Greek σπέρμα (seed) and ζῷον (living being) and more commonly known as a sperm cell, is the haploid cell that is the male gamete.

A mature human Spermatozoon

Spermatagonia divides several times during the process of sperm development. The entire process of sperm formation and maturation takes about 9-10 weeks. The separate divisions that take place and what happens in each are as follows:

- **First division**: The first division is done by mitosis, and ensures a constant supply of *spermatocytes*, each with the diploid number of chromosomes.
- **Second division**: Spermatocytes then undergo a series of two cell divisions during meiosis to become *secondary spermatocytes*.
- **Third division**: Secondary Spermatocytes finally become *spermatids*. Spermatids, which are haploid cells, mature slowly to become the male gametes, or *sperm*.

The sperm is the main reproductive cell in males. The sperms differ in that each carry a set of chromosomes dividing each into either a male, or female sperm. The females differ in that they carry a X gene, while the male sperm carry a Y gene. The female sperm also differ phenotypically in that they have a larger head in comparison to the male sperms. This contributes to the male sperm being lighter, and therefore faster and stronger swimmers than their female counterparts (although statistically there is still a 50% chance of an either XY or XX embryo forming.

Spermatozoan stream lines are straight and parallel. The tail flagellates, which we now know propels the sperm cell (at about 1-3 mm/minute in humans) by rotating like a propeller, in a circular motion, not side to side like a whip. The cell is characterized by a minimum of cytoplasm. During fertilization, the sperm's mitochondria gets destroyed by the egg cell, and this means only the mother is able to provide the baby's mitochondria and mitochondrial DNA, which has an important application in tracing maternal ancestry. However it has been recently discovered that mitochondrial DNA can be recombinant (WebMD, 2017).

Spermatozoa are produced in the seminiferous tubules of the testes in a process called spermatogenesis. Round cells called spermatogonia divide and differentiate eventually to become spermatozoa. During copulation the vagina is inseminated, the spermatozoa move through *chemotaxis* (see glossary) to the ovum inside a Fallopian tube or the uterus.

2.3.5. Sperm Pathway:

Spermatogenesis takes place inside a male's testes, specifically in the walls of the seminiferous tubules. The epididymis is a tortuously coiled structure topping the testis, it receives immature sperm from the testis and stores it for several days. When ejaculation occurs, sperm is forcefully expelled from the tail of the epididymis into the ductus deferens. Sperm travels through the ductus deferens and up the spermatic cord into the pelvic cavity, over the ureter to the prostate behind the bladder. Here, the vas deferens joins with the seminal vesicle to form the ejaculatory duct, which passes through the prostate and empties into the urethra. Upon the sperm's exit from the testes, into the vas deferens, muscular movements take over. When ejaculation occurs, rhythmic muscle movements of *peristalsis* propel the sperm forward. This continues throughout the remainder of the sperm's journey through the male reproductive system.

Sperm cells become even more active when they begin to interact with the *fertilizing layer* of an egg cell. They swim faster and their tail movements become more forceful and erratic. This behavior is called "hyper activation."

A recent discovery links hyper activation to a sudden influx of calcium ions into the tails. The whip-like tail (flagellum) of the sperm is studded with ion channels formed by proteins called CatSper. These channels are selective, allowing only calcium ion to pass. The opening of CatSper channels is responsible for the influx of calcium. The sudden rise in calcium levels causes the flagellum to form deeper bends, propelling the sperm more forcefully through the viscous environment.

3. MATERIALS & METHOD

- Green tea leaf extract
- Weighing balance
- Standard rat feed
- Standard rat cage
- Electronic weighing balance
- Hand gloves
- Dissecting kit
- Beakers
- Slides
- Microscope
- Litmus paper
- Oral canula
- Syringe

The green tea was purchased from Ogbeogonogo market, Asaba. A total of 20 mature male albino wistar rats weighing between 150-250g used for this study were procured from the Animal House of NnamdiAzikiwe University College of Health Sciences OkofiaNnewi campus.

3.1. PREPARATION OF GREEN LEAF TEA EXTRACT

5.0gm green tea was added to 100 ml of boiling water and was steeped for 15 min. The fusion was cooled to room temperature and was filtered. Tea leaves was extracted a second time with 100 ml of boiling water and filtered. Two filtrates were then combined to obtain a 2.5% tea aqueous extract (2.5 gm tea leaves/100 ml of water).

Similar procedure was performed with 10gms and 15gms green tea to prepare 5.0% aqueous green tea extract. The extract was then ready for oral administration.

3.2. ESTABLISHMENT OF ANIMAL MODEL

GROUPS	MATERIAL ADMINISTERED	WEEKS
CONTROL	Normal rat feed & water *ad libitium*	4 weeks
LOW DOSE	2.5gm/100ml 0f water	4 weeks
MEDIUM DOSE	5gm/100ml of water	4 weeks
HIGH DOSE	7.5gm/100ml of water	4 Weeks

Blood was collected via ocular puncture and centrifuged to obtain serum. The serum was assayed for testosterone using ELISA hormone test kit, there levels were recorded. The animals were sacrificed via cervical dislocation. The epididymis was carefully removed and cut to release the stored sperm for evaluation sperm parameters. Testis of each rat was dissected out and their weights were taken via electronic weighing balance

3.3. STATISTICAL ANALYSIS:

Using IBM SPSS Version 20. Analysis of variance (ANOVA) was used to compare means, and values were considered significant at P< 0.05. Post Hoc multiple comparisons for differences between groups within groups were established using least significant difference (LSD). With results presented as Mean ± S.E.M.

4. RESULTS

TABLE 1 COMPARISON OF NET GAIN OF BODY WEIGHT OF RATS TREATED WITH GTLE OF DIFFERENT DOSES AND RESPECTIVE CONTROLS. VALUES ARE AT MEAN ± SEM (IN %).

	MEAN±	SEM	P-VALUE
CONTROL (gm)	30.61 ±	0.102	
LOW DOSE (gm)	18.54 ±	0.034	.00
MEDIUM DOSE (gm)	12.63 ±	0.111	.00
HIGH DOSE (gm)	10.00 ±	0.102	.00

P-value is significant at p<0.05

There was a significant decrease in the body weight of the test group (18.54 ± 0.034, 12.63 ± 0.111, 10.00 ± 0.102), when compared to the control group; (p<0.05).

TABLE 2 COMPARISON OF TESTICULAR WEIGHT (GM %) BETWEEN CONTROLLED AND GTLE TREATED RATS

	MEAN ±	SEM	P-VALUE
CONTROL (GM %)	0.93 ±	0.068	
LOW DOSE (GM %)	0.89 ±	0.654	.05
MEDIUM DOSE (GM %)	0.85 ±	0.085	.01
HIGH DOSE (GM %)	0.77 ±	0.060	.00

P-value is significant at p<0.05

There was a significant decrease in the testicular weight of the medium and high dose test group (0.85 ± 0.085, 0.77 ± 0.060), when compared to the control group; (p<0.05). But there was no significant decrease in the testicular weight of the low dose test group (0.89 ± 0.654); (p>0.05).

TABLE 3 EFFECT OF GTLE ON SPERM COUNT IN CONTROL AND TREATED RATS

	MEAN ±	SEM	P-VALUE
CONTROL (10^6)	72.40 ±	0.511	
LOW DOSE (10^6)	68.54 ±	0.582	.12
MEDIUM DOSE (10^6)	61.10 ±	0.296	.03
HIGH DOSE (10^6)	47.40 ±	0.469	.00

P-value is significant at p<0.05

There was a significant decrease in the sperm count of the medium and high dose test groups (61.10 ± 0.296, 47.40 ± 0.469), when compared to the control group; (p<0.05). But there was no significant decrease in the low dose test group (68.54 ± 0.582); (p>0.05).

TABLE 4 Effect of GTLE ON SPERM MOTILITY IN CONTROL AND TREATED GROUPS

	MEAN ±	SEM	P-VALUE
CONTROL (%)	69.80 ±	0.426	
LOW DOSE (%)	63.54 ±	0.732	.01
MEDIUM DOSE (%)	58.40 ±	0.512	.00
HIGH DOSE (%)	52.60 ±	0.512	.00

P-value is significant at p<0.05

There was a significant decrease in the sperm motility of all test groups (63.54 ±0.732, 58.40 ±0.512, 52.60 ± 0.512); (p<0.05).

TABLE 5 EFFECT OF GTLE ON TESTESTERONE LEVELS

	MEAN ±	SEM	P-VALUE
CONTROL (ng/ml)	2.64±	0.119	
LOW DOSE (ng/ml)	2.43±	0.146	.23
MEDIUM DOSE(ng/ml)	2.01±	0.179	.02
HIGH DOSE (ng/ml)	1.61±	0.094	.01

P-value is significant at $p<0.05$

There was a significant decrease in the testosterone levels of the medium and high dose test groups (2.01± 0.179, 1.61± 0.094), when compared to the control; ($p<0.05$). There was no significant decrease in the low dose test group when compared to the control group; ($p>0.05$).

5. DISCUSSION

The study indicated a significant reduction in net gain of body weight with increasing dosage. This work agrees with previous work by (Sayana, *et al*., 2000). The study also showed a decrease in testicular weight.It has been discovered that, the cardinal antioxidative ingredient in the green tea extract is green tea catechins (GTC), which comprise four major epicatechin derivatives; namely, epicatechin, (EC), epigallocatechin (EGC), epicatechingallate (ECG), and epigallocatechingallate.It has been shown that the reduction of body weight after application of green tea extract may be due to inhibition of catechol-O-methyltransferase (COMT) enzyme by epigallocatechingallate (EGCG) of the green tea. This enzyme (COMT) is responsible for the degradation effect of nor epinephrine which can stimulate thermogenesis and is responsible for oxidation of fat (Sayana, *et al*., 2000).

Besides body weight reduction, high dose of tea extract can cause significant reduction in testicular weight in dose dependent manner. Weight of testis generally depends on the mass of spermatogenic cells. So it may be said that, testicular weight loss is due to the decreased number of spermatogenic cells. The study also showed a significant decrease in sperm motility and sperm count. This work also agrees with (Sayana, *et al*., 2000). The study also showed a decrease in testosterone levels which also agrees with (Sayana, *et al*., 2000).The decreased concentration of serum testosterone has also been reported earlier by green tea epigallocatechingallate (Adani, *et al.,* 2013). This reduced concentration of testosterone may be due to decreased activity of steroidogenic enzymes Δ5 3β HSD and 17β HSD (Chandra, *et al.,* 2011). Kao *et al.* (kao, *et al*., 2000) also reported the decrease in serum testosterone level after exposure of catechin in green tea. It has also been explained earlier that green tea extract polyphenols mainly EGCG has inhibitory effect on leydig cell testosterone production probably through cell signaling pathway, P-450 side chain cleavage and the function of 17β HSD (Figueiroa, *et al*., 2009).

5.1. CONCLUSION & RECOMMENDATION

The study showed that green tea leaf extract at relatively high dose may cause impairment of normal functional status of the testis in male wistar rats and thus its consumption at relatively high doses raises concern on male reproductive function in spite of its other beneficial effects.

REFERENCE

[1] Actis-Goretta L, Ottaviani JI, Fraga CG (2006) Inhibition of angiotensin converting enzyme activity by flavanol-rich foods. J Agirc Food Chem; 54: 229–34.

[2] Adani VM, Ahmad N, Mukhtar H. (2013) Molecular targets for green tea in prostate cancer prevention. J Nutr; 133: 2417S-2424S.

[3] Awoniyi DO (2010). The role of rooibos (aspalathus linearis), green tea (camellia sinensis) and commercially available rooibos and green tea antioxidant supplements on rat testicular and epididymal function. M. Sc. Faculty of health and wellness Sci., Cape Peninsula Univ. of Technology.

[4] Blom E (1983). Sperm Morphology with reference to bull infertility. Ludhiana, First All-India symposium on Animal Reproduction, pp.61-81.

[5] Balen AH, Conway GS, Kaltsas G, Techatraisak K, Manning PJ, West C, Jacobs HS (1995) Andrology: Polycystic ovary syndrome: the spectrum of the disorder in 1741 patients. Human Reproduct; 10: 2107-2111.

[6] Bhathena SJ, Velasquez MT (2002). Beneficial role of dietary phytoestrogens in obesity and diabetes. The American J. Clin Nutr; 76: 1191-1201.

[7] Chacko SM, Thambi PT, Kuttan R, Nishigaki I (2010). Beneficial effects of green tea: A literature review. Chinese Medicine; 5:13-21.

[8] Chaiyasut C, Kusirisin W, Lailerd N, Lerttrakarnnon P, Suttajit M, Srichairatanakool S (2011). Effects of phenolic compounds of fermented thai indigenous plants on oxidative stress in streptozotocin-induced diabetic rats. Evid Based Complement Altern Med; 2011:749307.

[9] Chandra AK, Choudhury SR, Neela D, Sarkar M (2011). Effects of green tea extract (Camellia Sinensis L.) on mophorlogical and functional changes in adult male gonads of albino rats. Indian j. of exp. biol; 49: 689-697

[10] Coimbra S, Castro E, Rocha-Pereira P, Rebelo I, Rocha S, SantosSilva A (2006). The effect of green tea in oxidative stress. Clin Nutr; 25(5): 790-796.

[11] Dacheux JL, Gatti JL, Dacheux F (2003). Contribution of epididymal secretory proteins for spermatozoa maturation. Microscopy Research and Technique; 61, 7-17 Divi RL, Doerge DR (1996). Inhibition of thyroid peroxidase by dietary flavonoids. Chem Res Toxicol; 9: 16–23.

[12] Dufresne CJ, Farnworth ER (2001). A review of latest research findings on the health promotion properties of tea. J Nutr Biochem; 12(7): 404-421.

[13] Dumitrescu E, Trif A, Argherie D, Cristina R.T (2009). The consequences in utero exposure to lead acetate on exposure and integrity biomarkers of reproductive system in female rats. Medicina Veterinara, XLII; (2): 295-300.

[14] Ehrmann DA (2005) polycystic ovary syndrome. New England J. Med.; 352: 1223-1236.

[15] El- Beltagy MA, Saleh SY, El-Ghannam AA, Ibrahim AI (2015). Protective Effect of Green Tea Extract on Heavy Metals-Induced Oxidative Testicular Damage in rats. Indian J. Appl. Res.; 5 (1): 577- 583

[16] Eldebaky HA, Sabra HA, Samy AA, Eman MG, El-Khadrawy HH (2015). Protective Effect of Green Tea (Camellia snensi) Extract on P53 Gene Mutation and Reproductive Toxicity in Male Rat Intl. J. Genet.; 5(2): 53-62

[17] Figueiroa MS, César Vieira JS, Leite DS, Filho RC, Ferreira F, Gouveia PS, Udrisar DP, Wanderley MI (2009). Green tea polyphenols inhibit testosterone production in rat leydig cells. Asian J Androl; 11: 362-370.

[18] Fran K, Donald E, James G (2000) Research trends in healthful foods. Food Tec.; 54 (10), 45–52.

[19] Frei B, Higdon JV (2003) Antioxidant activity of tea polyphenols in vivo: evidence from animal studies. J Nutr; 133(10): 3275-3284.

[20] Gomikawa S, Ishikawa Y, Hayase W, Haratake Y, Hirano N, Matuura H, Mizowaki A, Murakami A, Yamamoto M (2008). Effect of ground green tea drinking for two weeks on the susceptibility of plasma and LDL to the oxidation ex vivo in healthy volunteers. Kobe J. Med. Sci; 54(1): E62-72

[21] Guo Q, Zhao B, Li M, Shen S, Xin W (1996). Studies on protective mechanisms of four components of green tea polyphenols against lipid peroxidation in synaptosomes. Biochim Biophys Acta; 1304: 210–22.

[22] Gupta S, Saha B, Giri AK (2002). Comparative antimutagenic and anticlastogenic effects of green tea and black tea: a review. Mut. Res; 512: 37-65.

[23] Hassan EM, Kahilo KA, Kamal TA (2016) The Ameliorated Effect of Garlic and Green Tea on Lead Induced Testicular Toxicity in Rats Intl J. Sci. Res. (IJSR); 5 (5): 1927-1933

[24] Hassanzadeh BM, Emami SA, Mousavifar N, Esmaily HA, Mahmoudi M, Mohammadpoor AH (2013). Effects of Seeds Extract on Insulin Resistance in Women with Polycystic Ovarian Syndrome. Iran. J. Pharm. Res.; 12: 475-481.

[25] Horzic D, Komes D, Belscak A, Ganic KK, Ivekovic D, Karlovic D (2009). The composition of polyphenols and methylxantine in teas and herbal infusions. Food Chem; 115: 441-448.

[26] Jassem HM, Ismaiel HK, Jasem AY (2008). Effect of aqueous extract of green tea on sexual efficiency in adult male rats treated with alloxan. Iraqi J. of vet. sci; 22:75-82.

[27] Jiřina KA, Kováčik (2013). Effect of green tea extract on motility parameters of rabbit semen. J of Microbiol. Biotech Food Sci; 2 (Special issue): 1-13.

[28] Kimura M, Umegaki K, Kasuya Y, Sugisawa A, Higuchi M (2002). The relation between single/double or repeated tea catechin ingestions and plasma antioxidant activity in humans. Eur J. Clin. Nutr; 56(12): 1186.

[29] Kao YH, Hiipaka RA, Liao S. (2000) Modulation of endocrine systems and food intake by green tea epigallocatechingallate. Endocrinology; 141: 980-7.

[30] Lim DY, Lee ES, Park HG, Kim BC, Hong SP, Lee EB (2003). Comparison of green tea extract and epigallocatechin gallate on blood pressure and contractile responses of vascular smooth muscle of rats. Archives of Pharmacal. Res.; 26: 214-223.

[31] Ly C, Yockell-Lelièvre J, Ferraro ZM, Arnason JT, Ferrier J, Gruslin A (2014). The effects of dietary polyphenols on reproductive health and early development. Human reproduction update; dmu058.

[32] Maruyama K, Iso H, Sasaki S, Fukino Y (2009). The Association between Concentrations of Green Tea and Blood Glucose Levels. J Clin Biochem Nutr.; 44: 41-45.

[33] Moran LJ, Noakes M, Clifton PM, Wittert GA, Williams G,Norman RJ (2006). Short-term meal replacements followed by dietary macronutrient restriction enhance weight loss in polycystic ovary syndrome. The American J. Clin.Nutr; 84: 77-87.

[34] Morel I, Lescoat G, Cogrel P, Sergent O, Pasdeloup N, Brissot P, Cillard P, Cillard J (1993) Antioxidant and iron-chelating activities of the flavonoids catechin, quercetin and diosmetin on iron-loaded rat hepatocyte cultures. Biochem Pharmacol; 45: 13–9.\

[35] Namita P, Mukesh R, Vijay KJ (2012). Camellia Sinensis (Green Tea): A Review. Global J Pharmacol.; 6: 52-59.

[36] Neves ALA, Komesu MC, Di Matteo MAS (2010) Effects of green tea use on wound healing. Intl J. Morphol; 28: 905-910.

[37] Pastore R (2005). Green & White Tea Max: A Closer Look at the Benefits of Green and White Tea. Pastore formulations 2005. Paul DH (2008). "Effect of green tea polyphenols on Cadmium toxicity in rats" Dominican. Edu. J.; 6(2):1-10.

[38] Qureshi N, Sharma R (2012). Lead toxicity and infertility in female swiss mice: A review. J. Chem. Biol. Phys. Sci; 2 (4): 1849-1861.

[39] Rezk HM, Elsherbiny M, Elkashef WF, Taha M (2014). Effect of Green Tea Extract on the Interferon-Induced Testicular Apoptosis in the Adult Albino Rat: Immunohistochemical and Electron Microscopic Study. Reprod Syst Sex Disord; 3(4): 146. doi: 10.4172/2161- 038X.1000146

[40] Roshdy E, Rajaratnam V, Maitra S, Sabry M, Ait Allah AS, Al-Hendy A (2013) Treatment of symptomatic uterine fibroids with green tea extract: a pilot randomized controlled clinical study. Intl J of Women's Health; 5: 477–486 Sato KK,

[41] Sayana K, Lin S, Oguni I, Zheng G. (2000) Effects of green tea on growth, food utilization and lipid metabolism in mice. In Vivo; 14: 481-4.

[42] Sueoka R,Tanigaki H, Tajima, Nakabayashi A, Yoshimura Y, Hosoi Y(2010). Green tea extracts attenuate doxorubicin-induced spermatogenic

disorders in conjunction with higher telomerase activity in mice. J Assist Reprod Genet; 27:501–508

[43] Satoh K, Sakamoto Y, Ogata A, Nagai F, Mikuriya H, Numazawa M, Yamada K, Aoki N (2002) Inhibition of aromatase activity by green tea extract catechins and their endocrinological effects of oral administration in rats. Food Chem Toxicol; 40: 925–33.

[44] Sha'bani N, Miraj S, Rafieian-kohpayei M, Namjoo AR (2015). Survey of the detoxification effect of green tea extract on the reproductive system in rats exposed to lead acetate Adv. Biomed Res.; 4: 155

[45] Sharma RK, Agarwal A (1996). Role of reactive oxygen species in male infertility. Urology; 48: 835-850.

[46] Sheteifa MAM, Morsy WA (2014). Effect of green tea as dietary supplements (Camellia sinensis) on semen quality and testosterone profile in rabbits J. Animal and Poultry Prod., Mansoura Univ.; 5 (1): 1 – 13

[47] Shyamal Kanti Das, Soumendra Nath Karmakar (2015). Effect of green tea (camellia sinensis l.) leaf extract on reproductive system of adult male albino rats. Int J Physiol Pathophysiol Pharmacol; 7(4):178-184

[48] Tarek MH, Abdel-Tawab HM, Azza WI, Hala FA (2014). Oxidative damage and Reproductive toxicity associated with cyromazine and chlorpyrifos in male rats: The protective effects of green tea extract. Res. J. Environ. Toxicol.; 8 (2): 53-67.

[49] Tariq M, Naveed A, Barkat Ali K (2010). The morphology, characteristics, and medicinal properties of Camellia sinensis' tea. J. Med. Plants Res; 4(19): 2028-2033.

[50] Thuppil V, Tannir S (2013). Treating lead toxicity: possibilities beyond synthetic chelation. JKIMSU; 2 (1): 4-31.

[51] Valerie, C., Scanlon, & Tina, S. (2017). Human Physiology/The male reproductive system. In *Essentials of Anatomy and Physiology.* wikibooks.

[52] Vigorito C, Giallauria F, Palomba S, Cascella T, Manguso F, Lucci R, De Lorenzo A, Tafuri D, Lombardi G, Colao A (2007). Beneficial effects of a three-month structured exercise training program on cardiopulmonary functional capacity in young women with polycystic ovary syndrome. J. Clin Endocrinol. Metabolism; 92: 1379-1384.

[53] WebMD. (2017). Retrieved October 2017, from http://www.webmd.com

[54] Yang CS, Chung JY, Yang G, Chhabra SK, Lee MJ (2000). Tea and tea polyphenols in cancer prevention. J Nutr; 130: 472S–8S.

[55] Yousif WH, Adbullah ST (2010). Reproductive efficiency of rats whose mothers treated with lead acetate during lactation: role of vitamin E. Iraqi J. Vet. Sci; 24 (1): 27-34

[56] Zadkhosh N, Heidary ST, Ghafori KA, Mosavi L, Mehri N, Felegari H, Ranjbar A (2016). Protective role of green tea on malathion-induced testicular oxidative damage in rats. Asian Pacific J. of Reproduction; 5(1): 42–45.

[57] Zhang D, Al-Hendy M, Richard-Davis G, Montgomery-Rice V, Sharan C, Rajaratnam V, Khurana A, Al-Hendy A. (2010) Green tea extract inhibits proliferation of uterine leiomyoma cells in vitro and in nude mice. Am J Obstet Gynecol.; 202:e1–e9.